

What is Claimed is:

1. A method for inhibiting the expression of a target gene in a mammalian cell, the method comprising: introducing into the cell an RNA comprising a double stranded structure having a nucleotide sequence which is substantially identical to at least a part of the target gene and which is derived from an endogenous template; and verifying inhibition of expression of the target gene, wherein the mammalian cell is an oocyte, a cell of the early embryo or a somatic cell.
2. A method as claimed in claim 1, wherein the target gene is an endogenous gene.
3. A method as claimed in claim 1, wherein the target gene is a viral gene.
4. A method as claimed in claim 1, wherein the RNA is produced outside the cell.
5. A method as claimed in claim 4, wherein the RNA is injected into the cell.
6. A method as claimed in claim 1, wherein the RNA is produced within the cell.
7. A method as claimed in claim 1, wherein the RNA is produced recombinantly.
8. A method as claimed in claim 7, wherein the RNA is produced by an expression vector in the cell.
9. A method as claimed in claim 1, wherein the RNA comprises a single self-complementary RNA strand.
10. A method as claimed in claim 1, wherein the RNA comprises two separate complementary RNA strands.
11. A method as claimed in claim 1, wherein the nucleotide sequence is substantially identical to the whole of the target gene.

12. A method as claimed in claim 1, wherein the target gene causes or is likely to cause disease.

13. A method as claimed in claim 1, wherein the cell of the early embryo is an embryonic stem cell.

14. An RNA comprising a double stranded structure having a nucleotide sequence which is substantially identical to at least a part of a target gene in a mammalian cell and which is derived from an endogenous template for use as a medicament.

15. A mammalian cell containing an expression construct, the construct coding for an RNA which forms a double stranded structure having a nucleotide sequence which is substantially identical to at least a part of a target gene and which is derived from an endogenous template, wherein the mammalian cell is an oocyte, a cell of the early embryo or a somatic cell.

16. A method for attenuating expression of a target gene in cultured cells, comprising introducing double stranded RNA (dsRNA) into the cells in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

17. A method for attenuating expression of at least one target gene in cultured cells, comprising introducing at least one double stranded RNA (dsRNA) into the cells in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

18. A method for attenuating expression of at least one target gene in a mammalian cell, comprising introducing at least one double stranded RNA (dsRNA) into the mammalian cell in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

19. The method of claim 16, 17 or 18, wherein the double stranded RNA (dsRNA) hybridizes under stringent conditions to coding sequence of the target gene.
20. The method of claim 16, 17 or 18, wherein the double stranded RNA (dsRNA) hybridizes under stringent conditions to non-coding sequence of the target gene.
21. The method of any of claims 16, 17 or 18, wherein the target gene is an endogenous gene of the cell.
22. The method of any of claims 16, 17 or 18, wherein the target gene is a heterologous gene relative to the genome of the cell, such as a pathogen gene.
23. The method of any of claims 16, 17 or 18, wherein the cell is a primate cell, such as a human cell.
24. The method of any of claims 16, 17 or 18, wherein the dsRNA is at least 20 nucleotides in length.
25. The method of claim 18, wherein the dsRNA is at least 100 nucleotides in length.
26. The method of any of claims 16, 17 or 18, wherein expression of the target gene is attenuated by at least 10 fold.
27. A method for attenuating expression of a target gene in a cell, comprising introducing into the cell a hairpin nucleic acid in an amount sufficient to attenuate expression of the target gene, wherein the hairpin nucleic acid comprises an inverted repeat of a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.
28. A hairpin nucleic acid for inhibiting expression of a target gene, comprising a first nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene, and a second nucleotide sequence which is a complementary inverted repeat

of said first nucleotide sequence and hybridizes to said first nucleotide sequence to form a hairpin structure.

29. The method of claim 27 or the hairpin nucleic acid of claim 28, wherein the hairpin nucleic acid is RNA.

30. A double-stranded RNA for inhibiting expression of a mammalian gene, comprising a first nucleotide sequence that hybridizes under stringent conditions, including a wash step of 0.2XSSC at 65°C, to a nucleotide sequence of at least one mammalian gene and a second nucleotide sequence which is complementary to said first nucleotide sequence.

31. The double-stranded RNA of claim 30, wherein the first nucleotide sequence of said double-stranded RNA is at least 20 nucleotides.

32. The double-stranded RNA of claim 30, wherein the first nucleotide sequence of said double-stranded RNA is at least 25 nucleotides.

33. The double-stranded RNA of claim 30, wherein the first nucleotide sequence of said double-stranded RNA is at least 100 nucleotides.

34. The double-stranded RNA of claim 30, wherein the first nucleotide sequence of said double-stranded RNA is at least 400 nucleotides.

35. The double-stranded RNA of claim 30, wherein the first nucleotide sequence of said double-stranded RNA is identical to at least one mammalian gene.

36. The double-stranded RNA of claim 30, wherein the mammalian gene is a human gene.

37. The double-stranded RNA of claim 30, wherein the double-stranded RNA is a hairpin comprising a first nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of at least one mammalian gene, and a second nucleotide sequence which is a complementary inverted repeat of said first nucleotide sequence and hybridizes to

said first nucleotide sequence to form a hairpin structure.

38. The double-stranded RNA of claim 30, wherein the double-stranded RNA is an siRNA.

39. The double-stranded RNA of claim 30, wherein the first nucleotide sequence hybridizes under stringent conditions to a nucleotide sequencing corresponding to coding sequence of at least one mammalian gene.

40. The double-stranded RNA of claim 39, wherein the first nucleotide sequence is identical to a nucleotide sequencing corresponding to coding sequence of at least one mammalian gene

41. The double-stranded RNA of claim 30, wherein the first nucleotide sequence hybridizes under stringent conditions to a nucleotide sequencing corresponding to non-coding sequence of at least one mammalian gene.

42. The double-stranded RNA of claim 41, wherein the first nucleotide sequence is identical to a nucleotide sequencing corresponding to non-coding sequence of at least one mammalian gene

43. The double-stranded RNA of claim 41, wherein the non-coding sequence is a non-transcribed sequence.

44. A method for attenuating expression of a target gene in a non-embryonic cell suspended in culture, comprising introducing into the cell a double stranded RNA (dsRNA) in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.

45. The method of claim 44, wherein the target gene is an endogenous gene of the cell.

46. The method of claim 44, wherein the target gene is an heterologous gene relative to the genome of the cell, such as a pathogen gene.

47. The method of claim 44, wherein the cell is a primate cell, such as a human cell.
48. The method of claim 44, wherein the dsRNA is at least 20 nucleotides in length.
49. The method of claim 48, wherein the dsRNA is at least 100 nucleotides in length.
50. The method of claim 44, wherein expression of the target gene is attenuated by at least 10 fold.
51. A method for attenuating expression of a target gene in a cell, comprising introducing into the cell a hairpin nucleic acid in an amount sufficient to attenuate expression of the target gene, wherein the hairpin nucleic acid comprises an inverted repeat of a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene.
52. A hairpin nucleic acid for inhibiting expression of a target gene, comprising a first nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene, and a second nucleotide sequence which is an complementary inverted repeat of said first nucleotide sequence and hybridizes to said first nucleotide sequence to form a hairpin structure.
53. The method of claim 51 or the hairpin nucleic acid of claim 52, wherein the hairpin nucleic is RNA.
54. A nucleic acid comprising the following nucleotide sequences in a 5' to 3' order: an RNA polymerase promoter sequence; a first target sequence that is essentially complementary to a sequence of a target nucleic acid or complement thereof; a spacer sequence; a second target sequence that is essentially complementary to the first target sequence; and an RNA polymerase termination signal, wherein an RNA transcribed from the nucleic acid can inhibit expression of the target gene.
55. The nucleic acid of claim 54, wherein the RNA transcribed from the nucleic acid forms a

hairpin structure.

56. The nucleic acid of claim 54, wherein the first target sequence is at least about 95% identical to a nucleotide sequence of the target nucleic acid or the complement thereof.

57. The nucleic acid of claim 56, wherein the first target sequence is perfectly complementary to a sequence of a target nucleic acid or the complement thereof.

58. The nucleic acid of claim 56, wherein the target nucleic acid is a target gene.

59. The nucleic acid of claim 54, wherein the first and the second target sequences comprise from about 15 to about 30 nucleotides.

60. The nucleic acid of claim 59, wherein the first and the second target sequences comprise from about 19 to 25 nucleotides.

61. The nucleic acid of claim 54, wherein the first target sequence comprises a portion of the coding sequence of the target nucleic acid or the complement thereof.

62. The nucleic acid of claim 54, wherein the first and the second target sequences are perfectly complementary.

63. The nucleic acid of claim 54, which is DNA.

64. The nucleic acid of claim 54, which is in a plasmid.

65. The nucleic acid of claim 54, which is in an expression vector.

66. The nucleic acid of claim 65, wherein the expression vector is a eukaryotic expression vector.

67. The nucleic acid of claim 66, wherein the eukaryotic expression vector is a mammalian

expression vector.

68. The nucleic acid of claim 67, wherein the eukaryotic expression vector is a viral vector.

69. A cell comprising the nucleic acid of claim 54.

70. The cell of claim 69, which is a eukaryotic cell.

71. The cell of claim 70, which is a mammalian cell.

72. The cell of claim 69, which is an isolated cell.

73. A method for inhibiting the synthesis of a target protein in a eukaryotic cell, comprising introducing into a target cell a nucleic acid of claim 54 wherein the first target sequence is essentially complementary to a sequence of the nucleic acid encoding the target protein or the complement thereof, such that the nucleic acid is transcribed in the target cell and thereby inhibits the synthesis of the target protein.

74. A method for inhibiting the synthesis of a target protein in a cell of a subject, comprising introducing into the cell of the subject a nucleic acid of claim 54, wherein the first target sequence is essentially complementary to a sequence of the gene encoding the target protein or the complement thereof, such that the nucleic acid is transcribed in the target cell and thereby inhibits the synthesis of the target protein.

75. The method of claim 74, comprising first obtaining the cell from a subject; introducing the nucleic acid into the cell ex vivo and administering the cell to the subject.

76. A method for attenuating expression of a target gene of cells of a patient, comprising administering RNAi constructs formulated in liposomes and in an amount sufficient to attenuate expression of a target gene through an RNA interference mechanism, so as to thereby alter the growth, survival or differentiation of said cells.

77. The method of claim 76, wherein the RNAi construct is an small-interfering RNA (siRNA).

78. The method of claim 77, wherein the siRNA is 19-30 base pairs long.

79. The method of claim 76, wherein the RNAi construct is an expression vector having a coding sequence that is transcribed to produce one or more transcriptional products that produce siRNA in said treated cells.

80. The method of claim 76, wherein the RNAi construct is a hairpin RNA which is processed to an siRNA in said treated cells.

81. The method of claim 76, wherein the cell is a mammalian cell.

82. The method of claim 81, wherein the cell is a human cell.

83. The method of claim 76, wherein the liposomes are cationic liposomes including cationic vesicle-forming lipids.

84. A method of conducting a pharmaceutical business comprising: a) identifying an RNAi construct which inhibits proliferation of target cells in vivo and reduces the effects of a disorder involving unwanted proliferation of the target cells; b) conducting therapeutic profiling of the RNAi construct identified in step (a) for efficacy and toxicity in animals; and c) formulating a pharmaceutical preparation including one or more RNAi constructs identified in step (b) as having an acceptable therapeutic profile.

85. The method of claim 84, including an additional step of establishing a distribution system for distributing the pharmaceutical preparation for sale, and (optionally) establishing a sales group for marketing the pharmaceutical preparation.

86. A method of conducting a pharmaceutical business comprising: a) identifying an RNAi construct which inhibits proliferation of target cells in vivo and reduces the effects of a

disorder involving unwanted proliferation of the target cells; b) (optionally) conducting therapeutic profiling of the RNAi construct identified in step (a) for efficacy and toxicity in animals; and c) licensing, to a third party, the rights for further development of the RNAi construct.

87. A method to inhibit expression of a target gene in a cell comprising introduction of a ribonucleic acid (RNA) into the cell in an amount sufficient to inhibit expression of the target gene, wherein the RNA comprises a double-stranded structure with an identical nucleotide sequence as compared to a portion of the target gene.

88. The method of claim 87 in which the target gene is a cellular gene.

89. The method of claim 87 in which the target gene is an endogenous gene.

90. The method of claim 87 in which the target gene is a transgene.

91. The method of claim 87 in which the target gene is a viral gene.

92. The method of claim 87 in which the cell is from an animal.

93. The method of claim 87 in which the cell is from a plant.

94. The method of claim 92 in which the cell is from an invertebrate animal.

95. The method of claim 94 in which the cell is from a nematode.

96. The method of claim 87 in which the identical nucleotide sequence is at least 50 bases in length.

97. The method of claim 87 in which the target gene expression is inhibited by at least 10%.

98. The method of claim 87 in which the cell is present in an organism and inhibition of

target gene expression demonstrates a loss-of function phenotype.

99. The method of claim 87 in which the RNA comprises one strand which is self-complementary.

100. The method of claim 87 in which the RNA comprises two separate complementary strands.

101. The method of claim 100 further comprising synthesis of the two complementary strands and initiation of RNA duplex formation outside the cell.

102. The method of claim 100 further comprising synthesis of the two complementary strands and initiation of RNA duplex formation inside the cell.

103. The method of claim 87 in which the cell is present in an organism, and the RNA is introduced within a body cavity of the organism and outside the cell.

104. The method of claim 87 in which the cell is present in an organism and the RNA is introduced by extracellular injection into the organism.

105. The method of claim 87 in which the cell is present in a first organism, and the RNA is introduced to the first organism by feeding a second, RNA-containing organism to the first organism.

106. The method of claim 105 in which the second organism is engineered to produce an RNA duplex.

107. The method of claim 87 in which an expression construct in the cell produces the RNA.

108. A method to inhibit expression of a target gene comprising: (a) providing an organism containing a target cell, wherein the target cell contains the target gene and the target gene is expressed in the target cell; (b) contacting a ribonucleic acid (RNA) with the organism,

wherein the RNA is comprised of a double-stranded structure with duplexed ribonucleic acid strands and one of the strands is able to duplex with a portion of the target gene; and (c) introducing the RNA into the target cell, thereby inhibiting expression of the target gene.

109. The method of claim 108 in which the organism is an animal.

110. The method of claim 108 in which the organism is a plant.

111. The method of claim 108 in which the organism is an invertebrate animal.

112. The method of claim 108 in which the organism is a nematode.

113. The method of claim 108 in which the identical nucleotide sequence is at least 50 nucleotides in length.

114. The method of claim 108 in which the expression of the target gene is inhibited by at least 10%.

115. The method of claim 108 in which the RNA is introduced within a body cavity of the organism and outside the target cell.

116. The method of claim 108 in which the RNA is introduced by extracellular injection into the organism.

117. The method of claim 108 in which the organism is contacted with the RNA by feeding the organism food containing the RNA.

118. The method of claim 117 in which a genetically-engineered host transcribing the RNA comprises the food.

119. The method of claim 108 in which at least one strand of the RNA is produced by transcription of an expression construct.

120. A process for inhibiting gene expression in a cell comprising: delivering to the cell a combination of two or more RNA function inhibitors specific for the gene.

121. The process of claim 120 wherein at least one of the RNA function inhibitors induces RNA interference.

122. The process of claim 120 wherein at least one of the RNA function inhibitors consists of siRNA.

123. The process of claim 120 wherein at least one of the RNA function inhibitors is transcribed within the cell from a DNA expression cassette that is delivered to the cell.

124. The process of claim 123 wherein the expression cassette encodes an siRNA.

125. The process of claim 123 wherein the expression cassette encodes an antisense sequence.

126. The process of claim 120 wherein the combination of inhibitors consist of siRNA and antisense polynucleotide.

127. The process of claim 120 wherein the cell consist of an in vitro mammalian cell.

128. The process of claim 120 wherein the cell consists of an in vivo mammalian cell.

129. The process of claim 120 wherein inhibiting gene expression consists of providing a therapeutic effect.

130. The process of claim 129 wherein the gene consists of an infectious agent gene.

131. The process of claim 129 wherein the gene contributes to a disease state.